



## Neutrophil extracellular traps in ulcerative colitis

### *A proteome analysis of intestinal biopsies*

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rameters and their effect on DIA. Protein digest obtained from human cell line was analyzed in triplicates with different sample amounts from 250ng–2μg. DIA acquisition was obtained using MS scans analyzing 400-1000m/z with a resolution of 60k (400m/z). 30 MS/MS scans were acquired with 20m/z isolation window, loop count of 10 and orbitrap resolution of 30k (400m/z). For two fold change, we observed relatively better quantitative accuracy for lower protein amount compared to higher. For higher fold changes, we observed good quantitative accuracy irrespective of protein amount. Although DIA offers several advantages over DDA, informatics tools to deconvolute the data for identification and quantitation need significant improvement.

#### D-1-06:

##### **Targeted Interaction Analysis of FtsZ with Small Molecules and the Effect of Six Point Mutations of ClpX on BsFtsZ Polymerization**

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Drug resistance is the major concern for healthcare professionals and looking deep into the existing and new chemical libraries to explore the new drugs novel mechanism of action. Bacterial cell division process is highly conserved mechanism in prokaryotes and FtsZ is the key player involved in cytokinesis process along with many accessory proteins. Many natural compounds have showed potential antimicrobial activity by targeting the FtsZ polymerization. The protein expression profile of FtsZ has not changed much under curcumin, totarol and plumbagin treatment whereas mRNA expression analysis of FtsZ showed significant repression. In the present study, we have performed real-time interaction analysis of both BsFtsZ and EcFtsZ with curcumin, totarol and plumbagin for binding and kinetic analysis. Further, molecular docking has provided the putative binding sites for the small molecules in FtsZ. Besides, we also tested the effect of wild type and six point mutants ClpX on BsFtsZ polymerization. Interestingly, mutated ClpX has induced BsFtsZ polymerization in concentration dependent manner however wild type hasn't changed much. The real-time SPR analysis also showed that both wild type and mutated ClpX binds to BsFtsZ strongly in a concentration dependent manner. In summary, our study showed that the mentioned small molecules did not affect the FtsZ protein

expression levels rather bind with FtsZ at molecular level and regulate the FtsZ polymerization dynamics. This study also provided the KD for both BsFtsZ and EcFtsZ with the small molecules using SPR analysis. In addition, our results demonstrated that both wild type and mutant (six mutations) ClpX leads to inhibition of FtsZ polymerization with equal affinity. In depth analysis is necessary for conclusive prospective on role of mutated ClpX on bacterial cell division.

#### D-1-07:

##### **Neutrophil Extracellular Traps in Ulcerative Colitis—A Proteome Analysis of Intestinal Biopsies**

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Inflammatory bowel diseases, including Ulcerative colitis affects 2.5–3 million people in Europe. The life-long conditions significantly reduce the quality of life of the patients, and compose a significant economic burden for society. The etiology of these diseases remains incompletely explained. We, therefore performed the most thorough proteome-based study of ulcerative colitis colon tissue, based on number of analyzed proteins, and have for the first time observed neutrophil extracellular traps in ulcerative colitis, detected by proteomics and verified by microscopy.

We performed a comparative proteome analysis of non-inflamed mucosal colon biopsies from 10 patients with ulcerative colitis and 10 controls. The proteome of the biopsies were characterized by high throughput label-free quantitative proteomics, and the biopsy histology was analyzed by microscopy.

We quantified 5,711 different proteins in the colon biopsies. Forty-six proteins had a statistically significant difference in abundance between the ulcerative colitis colon tissue and controls. Eleven of the proteins with increased abundances in the ulcerative colitis biopsies were associated with neutrophils and neutrophil extracellular traps. The abundance of cal-

protectin and lactotransferrin in the tissue correlated with the degree of tissue inflammation as determined by histology. The findings were validated by microscopy where an increased abundance of neutrophils within the mucosal tissue was found. Additionally, we confirmed the presence of neutrophil extracellular traps by extracellular DNA in the ulcerative colitis colon tissue.

We found an increased abundance of several proteins hitherto not associated with innate immunity and neutrophils in non-inflamed mucosal colon biopsies from ulcerative colitis patients. The increased abundance of these antimicrobial compounds points to the stimulation of the innate immune system in the etiology of ulcerative colitis. Our findings demonstrate that even though remission has been achieved on the surface of the ulcerative colitis colon tissue, a chronic condition is still present within the morphologically normal tissue.

#### D-1-08:

##### Mass Spectrometric N-Terminal Sequencing of Peptides Using a Bacterial Aminopeptidase

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Aminopeptidases are exo-peptidases that catalyze the hydrolysis of peptide bonds joining the N-terminal amino acid of any peptide to the next amino acid in the sequence. Non-processive aminopeptidases are specific for certain residues and cannot progressively hydrolyze successively placed amino acid residues, whereas processive aminopeptidases tend to be non-specific and can 'progressively' cleave up to 30-35 amino acid residues in any peptide. Theoretically, therefore, beginning with a homogenous population of molecules of a defined mass constituting a single peptide of defined amino acid sequence, any processive aminopeptidase can be used to create a population of peptides of different lengths differing by one amino acid mass, so that the entire population can be studied in a single mass spectrum, to determine the peptide's amino acid sequence. However, in practice, it is difficult to control the reaction from progressing rapidly and also some peptides have blocked N-termini which prevent facile proteolysis. Sometimes, structure present in the peptide can also interfere. In this study, we describe the use of a non-specific, processive, deblocking *Bacillus subtilis*-derived aminopeptidase (BsuAP) in a reaction conducted at 70 degrees Centigrade for N-terminus sequencing.

Glu fibrino peptide (GFP B) from Waters, USA, was

used as substrate. GFP B is used as a standard (lock mass) in mass spectrometry and has a mass of 1570.67 Da. BsuAP was a recombinant protein expressed in, and purified from, *E. coli* in our own lab [3]. The reaction mixture of enzyme and substrate was prepared by mixing 1 nanomole of the aminopeptidase to 1.6 nanomoles of the substrate (GFP B) in water. The reaction mixture and control reaction (lacking aminopeptidase) were incubated at 70°C for 1 hour. After incubation, the reaction-mixture and control reaction were spotted on the MALDI plate. GFP B was used as a lock mass control. For spotting, 1 µl of sample was spotted with 1 µl CHCA matrix. Samples were analyzed on the Q-TOF Synapt G2S HDMS system (from Waters, USA), with laser desorption achieved by the instrument's 355nm laser. The spectra analyzed and presented are lock mass-corrected spectra.

The GFP B peptide is 14 residues-long. A sufficiently slow reaction would produce a mass spectrum containing the mass corresponding to the complete peptide, and all smaller masses progressively truncating the peptide from its N-terminus, assuming that the reaction does not proceed to completion on all substrate molecules. We found all expected masses down to 480.25 Da in the reaction spectrum. This data suggests that under the conditions used, BsuAP is non-specific and quite processive, progressively digesting 11 amino acid residues in the peptide substrate, beginning with the N-terminal glycine. Masses are seen to have different relative intensities which could be indicative of differences in specificity. It may be noted that the differences in masses of successive peaks (moving leftwards from the control GFP B mass) correspond to masses of the amino acids removed, after addition of the mass of one hydrogen atom.

#### D-1-09:

##### MRM Based Identification and Relative Quantitation of Vimentin in Different Grades of Meningiomas

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